INTERACTION AND FUNCTIONAL ASSOCIATION OF PROTEIN-DISULFIDE ISOMERASE WITH αVβ3 INTEGRIN ON ENDOTHELIAL CELLS

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Adhesive properties of endothelial cells are influenced by the thiol-disulfide balance but the molecular mechanism of this effect is unclear although recent observations indicate that integrin receptors may be a direct target for redox modulation. The purpose of this study was to examine whether protein-disulfide isomerase (PDI) is directly involved in this process. Since Mn^{2+} ions are known to affect the thiol-disulfide balance and activate integrins to maximal affinity we searched for protein-disulfide isomerase (PDI) interaction with integrins, particularly with αVβ3, in Mn^{2+}-treated endothelial cells.

To study of role of intramolecular thiol-disulfide exchange in the integrin molecule we used membrane impermeant sulfhydryl reagents p-chloromercuribenzene sulfonate (pCMBS) and 3-(N-maleimidylpropionyl)biocytin (MBP). By employing confocal microscopy and co-immunoprecipitation experiments was showed colocalization and physical association PDI and αVβ3 integrin on endothelial cells. The flow cytometry method were used for study effect of Mn^{2+} on the generation of free thiol groups on endothelial cell surface and in PDI and αVβ3.

We showed that exposure of endothelial cells to Mn^{2+} ions resulted in (a) appearance of surface protein thiol groups that can be found in PDI and αVβ3, and both proteins colocalize on the cellular surface, and (b) the formation of the PDI/αVβ3 stoichiometric complex, that dissociates upon reduction. The membrane impermeable sulfhydryl blockers (MPB, PCMBS), as well as the PDI inhibitors abolished binding of LM609 and vitronectin to endothelial cells activated by Mn^{2+}. Thus, we showed that the redox agent such as Mn^{2+} simultaneously modulates thiol isomerase activity of PDI that binds to αVβ3 and induces its transition to the ligand competent state, suggesting the alternative mechanism of integrin regulation.

In the present studies we showed that in Mn^{2+}-activated endothelial cells the surface-bound PDI is associated with αVβ3 and directly controls its ligand binding activity. Activation of the cells with Mn^{2+} ions leads to exposure of free sulfhydryl groups within αVβ3 and induces conformational changes in the context of the intact whole cell resulting in the increased ligation. Our data indicate that αVβ3 integrin is a substrate for PDI and generation of thiols and thiol-disulfide exchange appear to be mechanisms participating in the activation of integrins induced by Mn^{2+}.